

WHAT IS CLAIMED IS:

1. A method of modifying an initial antibody, the method comprising recombining a first nucleic acid or character string encoding an initial antibody of Table 1 or 2, or a homologue or fragment thereof, with one or more second character string or second antibody coding nucleic acid or second homologue or second fragment thereof, thereby producing a library of nucleic acids encoding modified antibodies, or a data set of nucleic acid character strings encoding modified antibodies.
2. The method of claim 1, wherein the recombining comprises recursively performing the steps of: recombining the first nucleic acid or character string encoding an initial antibody of Table 1 or Table 2, or a homologue or fragment thereof, with one or more second character string or second antibody coding nucleic acid or second homologue or second fragment thereof to produce a library of nucleic acids encoding modified antibodies, or a data set of nucleic acid character strings encoding modified antibodies, optionally performing a second recombination step in which the members of the library or the character strings in the data set are further recombined, selecting one or more resulting recombinant nucleic acids for a desirable trait or property, thereby producing first round selected nucleic acids or character strings and, performing a third recombination step in which the first round selected nucleic acids or character strings are recombined with each other, or with one or more additional nucleic acid or character strings.
3. The method of claim 1 or 2, wherein at least one of the first, second or additional nucleic acids encoding an initial antibody, or a homologue or fragment thereof further comprises a vector, which vector comprises an Ig expression cassette comprising: one or more cloning sites for heavy chain (VH) and light chain (VL) sequences; an encoded N-terminal fusion of VH and VL with an stII signal sequence; an encoded C-terminal fusion of VH and VL to human CH1 and a human CL regions; an encoded C-terminal fusion of VH/CL to a phage gIII protein; an amber stop codon at a CL/gIII border; and, a promoter selected from the group consisting of: a lacZ promoter, an alkaline phosphatase promoter, and an arabinose promoter.
4. The method of claim 2, further comprising selecting one or more resulting recombinant nucleic acids or character strings for a desirable trait or property.

5. The method of claim 4, wherein the trait or property is affinity maturation.
6. The method of claim 5, wherein the affinity maturation is ex vivo affinity maturation.
- 5 7. The method of claim 4, wherein the trait or property is increased affinity as compared to an antibody encoded by the first nucleic acid or character string.
8. The method of claim 2, further comprising performing recursive rounds of recombination with any of the enumerated nucleic acids or character strings, or with any products of any round of recombination.
- 10 9. The method of claim 1, wherein the recombination results in an improved nucleic acid which encodes at least one humanized antibody, which humanized antibody is humanized relative to the first nucleic acid.
10. The method of claim 1, wherein the recombination is performed in vitro, in vivo or in silico.
- 15 11. The library of nucleic acids produced by the method of claim 1.
12. One or more recombinant cells comprising one or more members of the library of claim 11.
13. A computer readable medium comprising the data set produced by the method of claim 1.
- 20 14. An Ig expression cassette comprising: one or more cloning sites for heavy chain (VH) and light chain (VL) sequences; an encoded N-terminal fusion of VH and VL with an stII signal sequence; an encoded C-terminal fusion of VH and VL to human CH1 and a human CL regions; an encoded C-terminal fusion of VH/CL to a phage gIII protein; an amber stop codon at a CL/gIII border; and, a promoter selected from the group consisting of:
- 25 a lacZ promoter, an alkaline phosphatase promoter, and an arabinose promoter.
15. The expression cassette of claim 14 wherein the cassette comprises a nucleic acid subsequence encoding a single-chain antibody (ScFv).

16. The expression cassette of claim 14, the vector comprising a human kappa CL or a human lambda CL.
17. The expression cassette of claim 14, wherein the stII signal sequence provides periplasmic targeting.
18. The expression cassette of claim 14, wherein the C-terminal fusions of VH/CL to the phage gIII protein provides for monovalent display.
19. The expression cassette of claim 14, wherein the amber stop codon produces soluble Fab in non-suppressing strains.
20. The expression cassette of claim 14, wherein the lacZ promoter provides inducible Ig gene expression in a host cell.
21. A recombinant cell comprising the vector of claim 14.
22. The cell of claim 21, further comprising one or more helper phage.
23. The cell of claim 22, comprising an M13KO7 phage or a VCS-M13 phage.
24. The cell of claim 21, which cell is a non-suppressing *E. coli* host.
25. The cell of claim 21, which cell is a non-suppressing *E. coli* host, strain W3110.
26. A library of recombinant cassettes comprising a plurality of antibody sequences, the library comprising a plurality of expression cassettes of claim 14, wherein the plurality of expression cassettes comprise a plurality of different antibody encoding nucleic acids.
27. The library of claim 26, wherein the different antibody encoding nucleic acids are shuffled variants of one or more antibody parental nucleic acid.

28. A method of evolving HIV envelope proteins with improved antigenicity, the method comprising:
- (a) providing a population of DNA fragments, which DNA fragments comprise at least one polynucleotide derived from at least one HIV envelope protein;
  - 5 (b) recombining the population of DNA fragments to produce a library of recombinant DNA segments;
  - (c) optionally repeating the recombining of steps (a) and (b) one or more times;
  - (d) screening the library of recombinant DNA fragments to identify at least one recombinant DNA segment encoding an evolved HIV envelope protein which has acquired or evolved
  - 10 a desired property;
  - (e) repeating the recombining of steps (a) through (d) until the evolved HIV envelope protein has acquired the desired property.

29. The method of claim 28, comprising providing a population of DNA fragments comprising at least one polynucleotide derived from at least one of an HIV gp120

15 and an HIV gp41 gene.

30. The method of claim 29, comprising providing a population of DNA fragments comprising a plurality of polynucleotides derived from at least one of an HIV gp120 gene and an HIV gp41 gene.

31. The method of claim 30, the plurality of polynucleotides being derived

20 from at least two clinical HIV isolates.

32. A library of recombinant HIV envelope protein genes produced by the method of claim 28.

33. The library of claim 32, the HIV envelope protein genes comprising a plurality of gp120 genes.

25 34. The library of claim 32, the HIV envelope protein genes comprising a plurality of gp41 genes.

35. An HIV vaccine comprising at least one polynucleotide selected from the library of claim 32.

36. The HIV vaccine of claim 35, comprising a plurality of polynucleotides.
37. The HIV vaccine of claim 36, the plurality comprising in excess of about 10,000 members.
38. An HIV vaccine comprising at least one epitope, which epitope is  
5 derived from an evolved HIV envelope protein produced by the method of claim 28.
39. The HIV vaccine of claim 38, wherein the evolved HIV envelope protein is an evolved gp120 protein.
40. The HIV vaccine of claim 38, wherein the evolved HIV envelope protein is an evolved gp41 protein.
- 10 41. The HIV vaccine of claim 40, wherein the epitope is a conformational epitope of gp41.
42. The HIV vaccine of claim 41, wherein the epitope is a gp41 resting state epitope.
43. The HIV vaccine of claim 42, wherein the epitope is a coiled coil.
- 15 44. The HIV vaccine of claim 41, wherein the epitope is a gp41 fusogenic epitope.
45. An antibody which binds at least one peptide encoded by the HIV vaccine of claim 35.
- 20 46. An antibody which binds at least one epitope of the HIV vaccine of claim 38.
47. The antibody of claim 45 or 46, wherein the antibody is a component of a polyclonal serum.
48. The antibody of claim 45 or 46, wherein the antibody is a monoclonal antibody.

49. The antibody of claim 45 or 46, wherein the antibody is encoded by a nucleic acid produced by at least one of recombination and mutagenesis.
50. The antibody of claim 49, wherein the antibody is encoded by a shuffled nucleic acid.
- 5 51. The antibody of claim 45 or 46, wherein the antibody is a humanized antibody.
52. The antibody of claim 51, wherein the antibody is a Fab fragment.
53. A reagent for the treatment or prevention of HIV infection, the reagent comprising at least one antibody of claim 45 or 46.
- 10 54. A reagent for the treatment or prevention of HIV infection, the reagent comprising a plurality of antibodies of claim 45 or 46.
55. A method of providing a population of recombinant anti-enterotoxin monoclonal antibody nucleic acids, the method comprising:  
hybridizing a set of overlapping anti-enterotoxin monoclonal antibody nucleic acid  
15 fragments; and,  
elongating the set of hybridized overlapping anti-enterotoxin monoclonal antibody nucleic acid fragments, thereby providing the population of recombinant anti-enterotoxin monoclonal antibody nucleic acids.
- 20 56. The method of claim 55, further comprising selecting one or more of the population of recombinant anti-enterotoxin monoclonal antibody nucleic acids for efficient binding by an encoded anti-enterotoxin monoclonal antibody to at least one enterotoxin.
57. The method of claim 55, wherein the set of overlapping anti-enterotoxin monoclonal antibody nucleic acid fragments is selected from one or more of: anti-*Staphylococcus* enterotoxin monoclonal antibody nucleic acid fragments and anti-*Streptococcus* enterotoxin monoclonal antibody nucleic acid fragments.  
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58. The method of claim 55, wherein the set of overlapping anti-enterotoxin monoclonal antibody nucleic acid fragments comprises one or more of: synthesized oligonucleotides and nuclease digested nucleic acids.
59. The population of recombinant anti-enterotoxin monoclonal antibody nucleic acids made by the method of claim 55.
60. The method of claim 55, further comprising expressing the population of recombinant anti-enterotoxin monoclonal antibody nucleic acids to provide at least one recombinant anti-enterotoxin monoclonal antibody.
61. The method of claim 60, wherein the at least one recombinant anti-enterotoxin monoclonal antibody is a polyreactive antibody.
62. The method of claim 60, wherein the at least one recombinant anti-enterotoxin monoclonal antibody is a hyper-reactive antibody.
63. The at least one recombinant anti-enterotoxin monoclonal antibody made by the method of claim 60.
64. The method of claim 55, further comprising introducing one or more of the recombinant anti-enterotoxin monoclonal antibody nucleic acids into at least one cell, wherein the one or more introduced recombinant anti-enterotoxin monoclonal antibody nucleic acids is expressed to provide at least one recombinant anti-enterotoxin monoclonal antibody.
65. The method of claim 64, wherein the at least one cell comprises a unicellular organism.
66. The at least one cell made by the method of claim 64.
67. The method of claim 55, the method further comprising:  
denaturing the population of recombinant anti-enterotoxin monoclonal antibody nucleic acids;  
rehybridizing the denatured population of recombinant anti-enterotoxin monoclonal antibody nucleic acids;

extending the rehybridized population of recombinant anti-enterotoxin monoclonal antibody nucleic acids to provide a population of further recombined anti-enterotoxin monoclonal antibody nucleic acids; and, optionally:

5       repeating the denaturing, rehybridizing, and extending steps at least once; and, optionally:

      selecting one or more of the population of further recombined anti-enterotoxin monoclonal antibody nucleic acids for efficient binding by an encoded anti-enterotoxin monoclonal antibody to at least one enterotoxin.

10       **68.** The population of further recombined anti-enterotoxin monoclonal antibody nucleic acids made by the method of claim 67.

**69.** The method of claim 67, further comprising expressing the population of further recombined anti-enterotoxin monoclonal antibody nucleic acids to provide at least one further recombined anti-enterotoxin monoclonal antibody.

15       **70.** The method of claim 69, wherein the at least one further recombined anti-enterotoxin monoclonal antibody is a polyreactive antibody.

**71.** The method of claim 69, wherein the at least one further recombined anti-enterotoxin monoclonal antibody is a hyper-reactive antibody.

**72.** The at least one further recombined anti-enterotoxin monoclonal antibody made by the method of claim 69.

20       **73.** A method for modifying the effector function of an antibody, the method comprising:

- (a) providing at least one nucleic acid derived from at least one immunoglobulin heavy chain constant region;
- (b) recombining the at least one nucleic acid to produce a library of recombinant  
25       immunoglobulin constant region nucleic acids;
- (c) optionally repeating the recombination process of steps (a) and (b) one or more times;
- (d) selecting at least one recombinant immunoglobulin constant region nucleic acid encoding a Fc region with a desired property;



(e) optionally repeating steps (a) through (d) one or more time until the Fc region has acquired a desired property.

74. The method of claim 73, comprising providing at least one nucleic acid encoding an antibody of Table 1 or Table 2.

5           75. The method of claim 73, comprising providing nucleic acids selected from a human, a non-human primate, a mouse, a rat, a hamster, a guinea pig, a rabbit, a cow, a sheep, a goat, a pig, a horse, a donkey, a camel, a chicken, a sequence that hybridizes to an immunoglobulin heavy chain region, or a sequence that encodes a protein that folds into an Ig-like domain.

10           76. The method of claim 73, comprising providing at least one nucleic acid, which nucleic acid comprises at least one of: a CH2 and a CH3 domain; a nucleic acid that hybridizes to a CH2 or a CH3 domain; a subsequence of a CH2 or a CH3 domain.

77. The method of claim 73, comprising selecting the at least one recombinant immunoglobulin constant region nucleic acid in vitro.

15           78. The method of claim 77, wherein the selecting is performed by an assay selected from: Fc receptor binding, complement fixation, complement mediated cell lysis, and activation of a proteolytic complement component, and flow cytometry.

79. The method of claim 73, comprising selecting selecting the at least one recombinant immunoglobulin constant region nucleic acid in vivo.

20           80. The method of claim 79, wherein the selecting is performed by an assay selected from: serum half-life, pathogenic challenge, toxin neutralization, small molecule clearance, half-life extension of a protein pharmaceutical, and tumorigenesis.

25           81. The method of claim 73, wherein the desired property is selected from among: Kd of Fc receptor binding, Kd of C1q binding, and activation of C1q proteolytic activity.

82. A method of humanizing an antibody, the method comprising:  
(i) selecting at least one non-human antibody with a desired antigen binding specificity;

- (ii) determining or inferring the amino acid sequence of the variable domain of the selected antibody;
- (iii) aligning a plurality of human antibody amino acid sequences with the amino acid sequence of the variable domain of the selected antibody;
- 5 (iv) providing oligonucleotides corresponding to at least one CDR domain of the non-human antibody and at least two corresponding variable domain framework regions of the human antibody sequences;
- (v) producing at least one library of synthetically shuffled humanized antibody sequences by randomly assembling the at least one CDR sequence with the at least two framework region
- 10 sequences maintaining amino acid positions relative to the plurality of human antibody amino acid sequences.

83. The method of claim 82, with the proviso that if a residue at a corresponding position differs between the mouse and human framework sequences, only oligonucleotides encoding the human residue are provided.

- 15 84. The method of claim 82, wherein at least one of the at least two corresponding variable domain framework regions of the human antibody sequences comprise wild-type human antibody sequences.

85. The method of claim 82, further comprising identifying at least one humanized antibody with a desired antigen binding specificity.

- 20 86. The method of claim 85, further comprising recombining the polynucleotide sequence encoding the humanized antibody with a desired antigen binding specificity with at least one additional polynucleotide sequence in vitro, in vivo, or in silico.

87. The method of claim 86, further comprising identifying at least one polynucleotide encoding a humanized antibody with a desired characteristic.

- 25 88. The method of claim 87, wherein the desired characteristic is selected from one or more of: affinity maturation, increased serum half-life, increased specificity, decreased immunogenicity, ???other properties to add.